ROMANIAN ACADEMY
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PhD. THESIS

SUMMARY

RESEARCH ON PARASITISM AND OTHER INTERSPECIFIC RELATIONSHIPS OF COLEOPTERA FROM DOLJ COUNTY

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CONTENTS

KEYWORDS

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intermediate host	**deuteronimpha**
definitive host	**predator**
ectoparasites	**development cycle**

INTRODUCTION

The present study entitled *Research on parasitism and other interspecific relationships of Coleoptera from Dolj County* synthetizes the results of the personal research related to the distribution and knowledge of Coleoptera species, as well as to the interspecific types of relationships with various organisms encountered within the ecosystems from Dolj County.

The thesis has 253 pages and is divided into 7 chapters, containing 24 tables and 131 figures (of which 72 original photos, 8 images taken from the specialized literature, 33 maps - 18 of which were made with Google Earth and 18 graphs and histograms). The bibliography includes 156 titles of works published both in the country and abroad and 69 online sources with various background information.

The achievement of the doctoral thesis pursued important objectives, which refer both to theoretical aspects (known from the specialized literature) and practical aspects (original research studies).

The knowledge of parasites and other types of interspecific relationships in Coleoptera involves a **theoretical** phase, which refers to the bibliographic documentation regarding their research at local, national and international level, a **practical** phase, which is represented by field trips for collecting host Coleoptera, and a **research** phase, in the laboratory, for identifying and determining parasites and other types of interspecific relationships.

At the beginning of the study, **theoretical** elements are represented by information including: a short history of the studies on the presence and research of Coleoptera species and of parasites and coleopteran predators in Dolj County; the Coleoptera order and factors influencing the insect life cycles; research methods used to collect the studied material; physico-geographic and morphological aspects of the research sites.

The **practical** elements that led to the personal contribution of this work include different aspects, such as the determination of Coleoptera species in Dolj County and the types of interspecific seasonal relationships between 2009 and 2017.

By elaborating this study, the intention was to synthesize, update and complete the data on the presence of some Coleoptera species in Dolj County and to report the presence of parasites, and not only, to the representatives of this group, based on the data from the specialized literature and on own research results.

In other respects, the aim of the study is outlined and completed by the determination of the specific composition, diversity, geographical distribution, ecological peculiarities and identification of certain species of parasites (and other categories of organisms) present at some species of Coleoptera from different ecosystems encountered in Dolj County. Moreover, this study also allows the development of certain principles for the conservation of their biodiversity.

A first chapter is dedicated to the history of the researches carried out in this field at local level, in the country and abroad.

The first mentions on the parasitic species of Coleoptera were made by Constantineanu M. I., in 1959, who mentioned them in volume 9 of the book Fauna R.P.R. (CONSTANTINEANU, 1959).

Later, in 1961, Panin S. together with Săvulescu N. achieved a more detailed study on the Cerambycidae family, also publishing under the aegis of the Romanian Academy Publishing House, Bucharest, the 5th fasciculus of volume 10 of Fauna R.P.R., in which the authors gathered data about the Cerambycidae coleopterans (longhorn beetles). Among other things, the authors mentioned the parasites and parasitoids known to date in Romania at these longhorn beetle species (PANIN & SĂVULESCU, 1961).

According to Balthasar, scarab beetles are host species for bacteria, protozoans, worms, nematodes, mites, Hymenoptera, and Diptera species (BALTHASAR, 1963).

Although we have not researched this issue, <u>we consider necessary to introduce the data provided by this author as they miss from the Romanian specialized literature</u>. Analyzing the information in this area, we have found out that a species can be host for various parasites or there are parasites specific to each species of scarab beetles.

In 1969, TUDOR CONSTANŢA, through the work *Parasite Chalcididae of Coleoptera* (Scolytidae and Cerambycidae), brings a new contribution to the knowledge of the chalcidoid fauna in our country, presenting 5 species from the families Eurytomidae, Eupelmidae and Pteromalidae. The hosts of these species were collected from the counties of Caraş Severin, Mehedinţi, Constanţa and Tulcea.

Important data are also given by the references to the presence of the species *Cerocephala trichotus* Ratzeburg, 1848, as a parasite of the larvae of Scolytidae coleopterans (*Hylesinus fraxini* Panz. and *Phloeosinus bicolor* Brull.) and of the species *Heydenia pretiosa* Forster 1856, which was discovered as a parasite of some Coleoptera: *Ips acumiatus* Gyll., *Hylesinus fraxini* Panz., etc., as well as of the curculionid *Magdalis armigela* Geoff. and the buprestid *Melanophila cyanea* F. (TUDOR, 1969).

Due to the fact that the parasite fauna of the Coleoptera was little studied in Romania and not at all in Dolj County, in order to have as complete documentation as possible, there were also consulted the publications of foreign authors, such as GYŐRFI, 1945 – 1947; BALACHOWSKY, 1962 – 1963; HURPIN, 1962 – for parasites and parasitoid species found at the following Coleoptera families: Anobiidae, Cerambycidae, Curculionidae, Melolonthidae, Rutelidae, Scarabaeidae and GRASSE 1953 and KUDO, 1966 – for protozoans (Protozoa, Sporozoa, Gregarinomorpha) that are parasites of Coleoptera.

As a conclusion, some Coleoptera are host species for bacteria, protozoans, fungi, nematodes, mites, Hymenoptera, and Diptera species. The consulted material allowed us to systematize only some of the information currently available in the country and abroad. On the other hand, the subject is not too much studied, interspecific relations as a subject of research thus becoming a real challenge.

In order to better understand how all these changes directly affect the development cycles of insects, especially of Coleoptera, we have introduced a chapter (**chapter 2**), which deals exclusively with the influence of environmental factors and their consequences on the studied group. All aspects are decisive, but the most important and decisive factor in the multiplication and perpetuation of coleopteran species is temperature.

Chapter 3 lists general data on the *Coleoptera order* (taxonomy and biology). Among the insects, the Coleoptera order is the largest and most widespread insect group on the globe, accounting for about 40% of the known insect species (IONESCU, 1962).

The ecology of Coleoptera presents highly varied aspects. There are terrestrial forms and aquatic forms. The diet of Coleoptera is extremely varied, as there are carnivorous, phytophagous, detritivore, mycetophagous, coprophagous, saprophagous species that rarely have a parasitic life.

As a result, although the beetles are the most numerous group, in the investigated ecosystems, their number was low due to the climate variations and changes occurred in the last years at local and global level. These oscillations and changes were obvious especially between 2009 and 2015, as there occurred very high temperature variations, frequent and heavy rainfalls, which are the determining factors for the reproduction, development and perpetuation of coleopteran species.

In **the fourth chapter**, after a general presentation of Dolj County (location, climate, relief, flora, fauna), there are rendered the physico-geographical and morphological characteristics of the researched ecosystems.

With regards to the Coleoptera biological material found in the field (782 specimens of which 16 specimens had various parasitic, predatory and phoretic forms), it was collected from two types of ecosystems: *aquatic* (Obedin Pool, Craioviţa Nouă Pool) and *terrestrial* (Bistreţ, Breasta - Obedin, Bâlta, Cernătești, Craiova, Bucovăţ Forest, Melinești – Ohaba, Mogoșești – Goești, Obedin, Radomir, Secui, Unirea – Risipiți, Urzicuța, Valea Stanciului).

All the ***16 settlements represent new collection and research sites*** for Dolj County and, implicitly, for Romania.

It is worth noting that the research sites Ciuperceni Noi, Bucovăţ-Leamna Forest, Ionele - Urzicuţa Lake, Radomir and Bistreţ have *a special status of protected areas of national interest* (natural reserves) located on the administrative territory of Dolj County, declared by Law no. 5 of March 6, 2000 (http: // apmdj.npm.ro/-/lista-ariilor-protejate-din-judetul-dolj).

Radomir Forest – the area was designated a ***Natura 2000 site*** according to G.D. no. 971/2011 for the modification and completion of G.D. no. 1284/2007 on the declaration of avifauna special protection areas as an integral part of the European ecological network Natura 2000 in Romania.

Bistreţ is a *protected area, a special avifaunistic protection area - SPA*, on the administrative territories of Bistreţ and Cârna settlements. The area was declared *Special Avifaunistic Protection Area* by the Government Decision no. 1284/October 24, 2007 (regarding the designation of special protection areas as an integral part of the Natura 2000 European ecological network in Romania) and covers an area of 1,916 hectares, including Bistreț Lake.

Bistreț Site (starting with July 2012) is protected by the ***Ramsar Convention*** as a wetland of international importance.

The diversity of coleopteran species and, not only, found and collected in the field, implied different collection methods rendered in a distinct chapter (**chapter 5**), in which we mentioned particular details for each method, both for the collection of coleopteran species, as well as for the collection, research and preparation of various categories of organisms found on/in Coleoptera. In order to accomplish this segment, I consulted in particular the following works: PANIN, 1957 and CHIMIŞLIU, 2001.

During the research, the collection of Coleoptera was made seasonally, in different periods of each year, starting from March till September. With regard to the collection methods, besides to the necessary equipment (entomologic jar, dissection kit, Olympus Bx43 stereo microscope, U-DA 1K50819, Olympus BX 43 optical microscope with camera, Panasonic camera, Lumix camera, Samsung Camera, tweezers, netting, plastic / paper bags, blades, lamellae, Petri dishes, platinum loop, reagents, etc.), for the correct diagnosis of parasites, predators and mites, it is also necessary to respect some stages related to the *epidemiological* and *clinical* aspects and to the *laboratory examinations*; it is about the *visual macroscopic examination* of the coleopteran and its body surface for the detection / presence of parasites, predators and / or mites; then, it is the *microscopic examination* of the fresh lamella biological material, but also the *stereomicroscopic examination* in incident and reflected light of the parasitized anatomical parts.

The **practical** and **research** elements that represent the personal contribution to this work are rendered in **Chapter 6**.

Interspecific relationships within a biocoenosis or an ecosystem are extremely complex, constituting a true network of causes and effects (CIOBOIU-CODOBAN, 2005).

Having as main purpose the identification of interspecific relationships, we carried out an analysis of these in relation to the collected coleopterans and we identified two main categories:

- interspecific relationships established according to the direct effect criterion - unilaterally positive and unilaterally negative relationships (+ -), respectively ***parasitism relationships and predatory relationships***;
- interspecific relationships established on the basis of their role in the life of populations: relationships related to distribution – ***phoretic*** or ***transport***.

In a **first subchapter** we analyzed the distribution of the coleopteran species, as well as of some of their parasites and predators, in Dolj County and the neighbouring areas. For its compilation, in accordance with the nomenclature and taxonomy used in Fauna Europaea (2009-2017) and the existing data in the previously published literature, we organized and presented the information material on the distribution of Coleoptera and their parasites, predators and mites in the fauna of the Dolj County from a systematic and ecological point of view (Bobîrnac (1960; 1967); Bobîrnac *et al.* (1964, 1966, 1968; 1982; 1999a); Bobîrnac (1970, 1974, 1973; 1975; 1960); Bobîrnac & Matei (1983, 1985a and 1985b); Bobîrnac & Sanda (1964); Botu, 1998; Chimişliu (2000a, 2000b, 2000c, 2000d, 2000e); Chimişliu (1999; 2001a; 2001c; 2002; 2003; 2004; 2008); Chimişliu *et al*., (2002; 2004); Chimişliu & Mogoşanu (2011); Firu (1982); Fleck (1904); Ilie & Chimişliu (2000); Ieniştea (1975); Kuthy, 1900; Ieniştea, 1975; Marcu (1928); Matei (1974; 1975a; 1976); Matei et al. (1975a, 1975b, 1976, 1978); Matei *et al.* (1974 - 1978); Marcu, 1928; Nieto & Keith, 2010; Neţoiu & Chimişliu (2004); Pisică, 2001; Pisică & Popescu, 2009; Panin & Săvulescu, 1961; 1969; Ruicănescu (1997; 1992a); Ruicănescu & Patko, 1995; Săvulescu, 1969; Serafim, 1985; Serafim *et al*., 2004; Serafim *et al*., 2004; Serafim, 2009, 2010; Serafim & Chimişliu, 2009) and added data on the species collected and examined between 2009 and 2017 (Lila, 2017a, b; Lila, 2015a, b; Lila, 2012).

For data processing to be concrete and useful, we also analyzed the material from the viewpoint of the diversity of host coleopterans in which the parasite species are present. 67 species of Coleoptera were found, which as hosts, belong to three superfamilies: Scarabaeoidea, Chrysomelidae, Dytiscidae; most of them belong to the Scarabaeoidea superfamily. Each one is host to several species of parasites and predators.

At the same time, there were also drawn maps containing the previous reports in the fauna of Dolj County, but also the collected and examined material where we found parasites, predators and mites.

In the **next subchapter** we summed up the results of the original researches on the types of interspecific relationships at Coleoptera.

The research was carried out by macroscopic examination, according to the established rules of ecological parasitology, aiming at: the detection of parasites on / in fresh biological material, the number of all parasites detected on each host coleopteran, as well as the correlation between parasites and their living environment. The samples were also examined microscopically as fresh and colored smear (Giemsa, malachite green), as well as stereomicroscopically through transparency.

The 16 identified parasites belong to 2 kingdoms, 5 phyla, 10 classes, 10 orders, 9 families, 9 suborders.

The taxonomic classification of the coleopterans was achieved according to Panin, 1955, 1957; Panin & Savulescu, 1961; Reitter, 1908–1916; Berlese, 1903; Bregetova & Koroleva, 1960; Costa, 1963, 1964, 1966; Gisela Rack, 1984, but also by accessing online web files that refer to the taxonomy and biology of parasites, predators and mites.

The ***taxonomy*** and ***nomenclature*** of the identified species is made according to Fauna Europea database (2009-2017) in most cases. The species of Coleoptera are presented in a systematic order and each of them includes the scientific and popular names, the identified species of parasites, predators and mites (with the scientific name), research sites and collection date. Moreover, for each locality where the collection was made, there are also rendered the geographical coordinates and flora and fauna information.

In order to have complete information, there were achieved maps where we marked the collection sites reported before the study and during the study period (2009-2017) for the investigated coleopteran species. In the same context, we mentioned the species of parasites, predators and mites that were encountered over time, but not identified in the present study.

The main purpose of the research was to show what coleopteran species are present in Dolj County and what type of interspecific relations are specific to them.

The hosts, from a systematic point of view, belong to the Order Coleoptera, more specifically to 7 families: Aphodiidae, Cerambycidae, Chrysomelidae, Dynastidae, Dytiscidae, Melolonthidae, and Scarabaeidae.

According to the collected and analysed data, the following taxa were reported for Coleoptera: 2 suborders (Adephaga and Polyfaga); three superfamilies (Caraboidea, Cucujiformia, Scarabaeiformia), seven families (Dytiscidae, Cerambycidae, Chrysomelidae, Aphodiidae, Dynastidae, Melolonthidae, Scarabaeidae), seven subfamilies (Dytiscinae, Cerambycinae, Chrysomelinae, Aphodiinae, Dynastinae, Melolonthinae, Scarabaeinae), six tribes (Cybistrini, Oryctini, Pentodontini, Melolonthini, Coprini, Onthophagini), nine genera (*Cybister*, *Cerambyx*, *Melasoma (Chrysomela), Aphodius*, *Oryctes*, *Pentodon*, *Melolontha*, *Copris, Onthophagus*), three subgenera (*Scaphinectes, Chrysomela, Palaeonthophagus*) and 10 species.

With regard to the classification of the coleopteran species found and collected in the field, the results are varied:

- aquatic coleopterans (*Cybister lateralimarginalis*);
- saproxylic coleopterans, indicators of forests of European importance (*Orictes nasicornis*),
- saproxylophagous coleopterans, species threatened with extinction *(Cerambyx cerdo),*
- defoliating coleopterans (*Melasoma populi*),
- coprophagous coleopterans (*Aphodius fimentarius*, *Copris lunaris*, *Onthophagus* (*P.*) *vacca, Onthophagus taurus*), the most frequent ones.

Entomopathogenic fungi of the *Deuteromycota* group (Fungi: Ascomycota: Sordario-mycetes: Hypocreales: Cordycipitaceae: *Beauveria* and *Metarhizium*) produce diseases called muscardine diseases.

Beauveria bassiana produces **white muscardine** at the larvae and adults of Scarabaeidae and other species, while *Metarrhizium anisopliae* produces **green muscardine.**

a) ***Beauveria bassiana* microfungus** is a very interesting controlling agent as it can infect the host by ingestion or by simple contact, unlike other microbiological control agents. This mode of action affects all sensitive stages (egg, larvae, adult).

After the macroscopic examination, we took a sample from the insect body by means of a platinum loop and distributed it on a suitable solid culture medium in a Petri dish.

Beauveria bassiana was identified at *Cerambix cerdo*. The fungus appears all over the world. Like any microorganism, *B. bassiana* has the potential to act as an **opportunistic pathogen agent** and appears on the most common culture media. It produces many conidia, like a powder, in distinct balls of white spores, as it was the case at *C. cerdo*. It rapidly colonizes the host organism, killing it in a few days, depending on the environmental conditions.

Metarhizium anisolpliae is reported to be able to infect more than 100 different insect species belonging to a very large variety of insect orders. The hosts are mainly insects belonging to Lepidoptera, Coleoptera and Homoptera.

The rapid production of destruxins in larvae causes their death. *M. anisopliae* also produces toxic proteolytic enzymes (MARK et al., 1989).

Among the industrial preparations based on entomopathogenic fungi, we exemplify: *Muscardin* and *Beauveria* – both based on the fungus *Beauveria baessiana*.

Acanthocephala (Acanthocephala: Archiacanthocephala: Oligacanthorhynchida: Oligacanthorhynchidae) are represented in our study by ***Macracanthorhynchus hirudinaceus*.**

Acanthocephala phylum is a taxonomic group of acoelomate forms that includes more than 1,000 species endoparasitic in the intestines of different invertebrate and vertebrate species, and as larvae in the body of the arthropods.

Macracanthorhynchus hirudinaceus (Pallas 1781) is a parasite characteristic to swine, but sometimes it affects people as well. In its development, it has two hosts (BALTHAZAR, 1963; MOWLAVI *et al*., 2006): *the intermediate host* – a cockchafer larva (*Melolontha melolontha*) or a rose chafer larva (*Cetonia aurata*), which live in the soil and the *definitive host* - the pig, accidentally the human being (CUCORANU, 2010).

It was prepared a smear with segments from the general cavity of the beetle body, which was exposed to an Olympus Bx43 Stereomicroscope, U-DA 1K50819. It was identified a 80-100 μm long and 50 μm wide egg, ovoid, dark brown, textured, displaying an outer hard capsule. It contained a larva (acanthor) with hooks. When it is ingested by the definitive host (pig, dog, etc.), the hooks will open and fix on the wall of the intestine, triggering adult development and frequently causing acanthocephaliasis, spread around almost the whole globe, which then evolves chronically, with digestive disturbances, or asymptomatically. People can get infected by ingesting an infected intermediate host, a beetle.

The parasite was signalled at *Scarabeus* sp., *Melolonta* sp., *Hydrophylus* sp. (BALTHASAR, 1963) and *Cetonia aurata* (CUCORANU, 2010). The result of the research brings something new in terms of the presence of *M. melolontha* parasite.

Mites (Animalia: Arthropoda: Chelicerata: Arachnida: Acari) continuously reproduce in an appropriate environment, but they disperse when certain environmental factors adversely affect their presence in the habitat (KRANTZ, 1999). The specialization of the dispersed behaviour reveals the variability that contributes to the distribution and diversity of mites (MITCHELL, 1970 *In*: TATYANA SACCHI & PIRES DO PRADO, 2004).

The particularities of phoresy (transport of mites from one place to another with the help of another organism) include active host search, recognition of attachment signs and host specificity, tranquility, recognition of host abandonment signs and, if necessary, synchronization with the life cycle of the host. The recognition of the ideal host is fundamental for transportation to a new location and is often based on chemical or olfactory stimuli produced by the host.

Phoresy may be an adaptation for survival or it may be a parasitic manifestation as it involves displacement through interactions within the ecosystems.

Environmental variations, with their effect on intra and interspecific relationships, lead to displacement and persistence in different habitats. The difficulty in defining phoresy reflects the diversity of behavioral and ecological parameters of the involved species.

Phoresy is important for the maintenance of the species that may act as predators or parasites during different development stages and it serves to define the survival strategy of the mutualistic predatory and parasitic species (TATYANA SACCHI & PIRES DO PRADO, 2004).

In the present study, mites are represented by 6 species belonging to one order (Mesostigmata) and three families (Macrochelidae, Uropodoidea, Laelapidae).

Uropodoidea **mites** are represented in this study by *Uropoda copridis* (Arachnida: Acari: Parasitiformes: Mesostigmata: Uropodoidea: Uropodidae) and *Uropoda* sp. identified at the females of the coprophagous coleopteran *Copris lunaris*, collected from different locations.

The macroscopic examination led us to the conclusion that a total of 52 individuals were found on the ventral side of the abdomen and on the feet of the *Copris* female. Most of them were located on the ventral side of the abdomen (36), the others being asymmetrically located, some grouped, on the three pairs of legs.

Subsequent visualization at the stereomicroscope showed that the identified mite species is *Uropoda copridis*. All specimens of this species were in the phoretic stage, deutonymph.

In case of Coprinae, the fact that adults take care of the nymphs gives mites the rare access to both generations: parents and offspring (MAŠÁN & HALLIDAY, 2009).

It is interesting to notice the female model known only by *Uropoda copridis*, originally discovered at a larva in a *Copris lunaris* ball and described by Mašán & Halliday. It suggests another type of dispersion strategy as it seems that the *Uropoda copridis* deutonymph does not attach to a new individual until it emerges from the brood ball (MAŠÁN & HALLIDAY, 2009). The deutonymph of *U. copridis* attaches to the host using an anal uropod pedicel, as it is found in many species of Uropodina (ATHIAS-BINCHE, 1984 *In*: MAŠÁN & HALLIDAY, 2009).

U. copridis showed a strong preference for parental beetles, for females in our study, instead of brood balls. This behavioral detail confirms the specialized observations - ATHIAS-BINCHE, 1984 *In*: MAŠÁN & HALLIDAY, 2009.

A more detailed study of the life cycles and behavior of these mites would be a valuable addition to our understanding of the ecology of the arthropods in this category, with practical implications for biodiversity conservation.

Macrocheles muscaedomesticae (Arachnida: Micrura: Acari: Anactinotrichida: Mesostigmata: Dermanyssina: Eviphidoidea: Macrochelidae).

The species was identified at *Pentodon idiota idiota*. The macroscopic examination helped us identify the mites attached to the ventral side of the body. Later, using the stereomicroscope, all the mite specimens were visualized, all of them being in the deutonymph stage.

Macrochelidae are a cosmopolitan family of predatory mesostigmatic mites, many of which occupying specialized and often unstable habitats. Most of the well-known species adapted to live in garbage dumps where the prey is abundant, this being a favouring factor for a rapid population growth.

As it regards their presence at Coleoptera, the specialized literature provides brief information. In the country, there were reports for the presence of *Macrocheles punctillatus* (Willm.), *Macrocheles plumiventris* Hull and *Anoetus ferroniarum* (Duf.) at the species of the genus *Onthophagus* latr.; *Macrocheles glaber* Müll and *Anoetus ferroniarum* (Duf.) at *Aphodius* Illig. (BALTHASAR, 1963).

The olfactory receptors on the tarsi allow mites to find their hosts, while the receptors on the top are involved in the localization and attachment to the host and in the perception of the substrate during motion (FARISH & AXTELL, 1966; WICHT *et al.*, 1971; COONS & AXTELL, 1973; HUNTER & ROSARIO, 1988 *In*: TATYANA SACCHI *et al.*, 2004).

The mite *Hypoaspis* sp. (Arthropoda: Arachnida: Acari: Mesostigmata: Laelapidae) was identified in our study at an *Orictes nasicornis* female.

It is difficult to draw firm conclusions about the specificity of the host because the studies conducted in this direction worldwide are brief, while at national and local level, there are no studies at all. In the notes of Joharchi & Shahedi for Iran, almost all the identified species of *Hypoaspis* sp. are associated with Coleoptera, especially with the species from the Scarabaeidae family, while few were collected in the soil (JOHARCHI & SHAHEDI, 2016). *Hypoaspis rhinocerotis* Oudemans feeds on the eggs of the scarab *Oryctes rhinoceros* L., a pest of the coconut palm.

Hypoaspis athiasae Costa, originally associated with *O. monocerus* Oliver, from Côte d'Ivoire, feeds on *O. rhinoceros* eggs (COSTA, 1971; GERSON *et al*., 2003 *In:* MOHAMMAD KHANJANI *et al*., 2013). In addition to *Hypoaspis athiasae*, Costa (1971) described five more species collected from Coleoptera in Israel, Côte d'Ivoire and Western Samoa. Khanjani & Ueckermann (2005) described *Hypoaspis polyphyllae* from *Polyphylla olivieri* (*In:* MOHAMMAD KHANJANI *et al*., 2013).

This study draws attention to the existence of the mite species and opens a research section for future years. In the specialized literature published in the country, we have not found any mentions of *Hypoaspis* sp. at *Orictes nasicornis.*

The dominant species belongs to the genus *Macrocheles* sp. and was identified at *Pentodon idiota idiota*, *Onthophagus vacca* (2 specimens) and *O. taurus*, new records for Dolj County and Romania.

The predatory pentatomidae *Zicrona caerulea* (Heteroptera: Pentatomoidea: Pentatomidae: Asopinae) was identified at a larva of a defoliating coleopteran – *Melasoma (Crysomela) populi.*

By macroscopic observation we found out that it was in the immature stage, the final stage of development, nymph L3, a predatory form.

These bedbugs are predators for poplar beetles, larvae of various coleopterans and moth caterpillars, and they also feed on plants. In the UK and / or Ireland it is considered a predator of the adults of *Altica* sp. and *Galerucella* sp. and this aspect is exploited (URBAN, 2006).

Following this study, the pentatomidae *Z. coerulea* is considered to be very useful, being reported as a predator of the larvae of *M. populi*, a defoliator coleopteran; it frequently contributes to the destruction of the larval stage of the chrysomelid. We consider it to be a natural solution that must be exploited to achieve a balance in the ecosystem.

The nematode *Ascarops strongylina* (Spiruroidea: Spirocercidae). During the study, it was found in a male specimen of the coprophagous *Aphodius fimetarius*, as an intermediate host. By microscopic examination there were identified eggs in the infectious stage (L3) in the Malpighian tubule system.

The eggs are eliminated with feces and consume their larval stages in the adults of the coprophagous coleopterans *Aphodius* sp., *Onthophagus* sp. or *Gymnopleurus* sp. (BALTHAZAR, 1936). The infectious stage of the parasite (L3) develops inside the beetle and, according to some authors, in birds (ODENING, 1968 – 69 *In*: BEN DAWES, 1976). Then, it is ingested by the pig together with the water or the food it consumes (OLSEN, 1974) and it reaches the wall of the stomach lining (MEHLHORN, 2008; KUMAR, 2004) or the small intestine, the pig being the definitive host.

The endoparasitic nematode *A. strongylina* was reported in the country in the species of *Aphodius* genus, but this research signals it for the first time for Dolj County as the infectious stage of the parasite (L3) in *A. fimentarius* as an intermediate host.

By macroscopic observation, **the Mermithidae *Drilomermis leioderma*** (Adenophorea: Enoplea: Enoplia: Mermithida: Mermithidae) was identified as ectoendoparasite in the larva of the aquatic coleopteran *C. lateralimarginalis*.

Unlike tetradonematid specimens, the adults of which develop in host insects, the Mermithidae representatives are not normally found as adults within the cavities of the insect body. They always leave the insect in the last larval stage, called post-parasite larva. The appearance is needed for adult development.

The post-parasite Mermithidae larva is equipped with a lance type tooth that is used to perforate the cuticle of the insects from inside. The hole in the body of the insect caused by the presence of this Mermithidae usually determines the insect death due to the loss of body fluids. The insect does not feed and is dependent on the food stored in the trophosome.

This study introduces into the information circuit a new sign of the Parasite larva of *Cybister lateralimarginalis*. The specialized literature indicates that the nematode *Drilomermis leioderma* is also described in *Cybister fimbriolatus* (Say) larvae (Dytiscidae: Coleoptera) in Louisiana (POINAR & PETERSEN, 1978).

Nematomorphs are a group of parasitic worms that develop in their hosts (especially terrestrial insects, arthropods), but reproduce in aquatic environments. **Horsehair worms** or **Gordius worms** (Nematomorpha: Gordioidea: Gordiidae) have been identified so far with 22 genera (Gordiida) and 5 marine species (Nectonema) during the developing juvenile phase, having parasitic activity in arthropods (HONG EUI-JU *et al.*, 2016).

Macroscopic research and subsequent stereomicroscopic visualization of the preparation allowed the identification of the nematode *Gordius aquticus* in *Melolontha melolontha*. The parasite stage is the final one (the larva transformed into an adult) and the coleopteran *M. melolontha* is the final host that shows *periodic larval* or *neotelian parasitism*.

It seems that the *Gordius* larvae swim freely in the water after hatching and are ingested when insects drink water. Once it has entered a host insect (by piercing or being swallowed), the larva penetrates the intestine of the insect and enters its cavity.

These will develop and will emerge from the body of the host as adults through the penetration of the body or through the anus after a few weeks. These larvae have no digestive tract and absorb their food through the skin (osmosis) (FERDA PERÇIN-PAÇAL & SERAP SANCAR-BAŞ, 2008).

They mature and leave the host after a while. Thorne (1940) suggested that adulthood occurs only in the presence of water, although the mechanism for detecting water is not known. THOMAS *et al.* (2002) showed that infected hosts are more likely to enter water than uninfected hosts (*In*: CAPINERA, 1999).

The studies regarding the behavior of this parasite have shown that host manipulation tactics have chemical bases. The parasite produces certain effector molecules that manipulate the central nervous system of the host to behave differently than normal, such as jumping into the water. In addition, the parasite produces a group of proteins of Wnt family that are similar to insect proteins, suggesting **molecular mimicry** as a possible source of behavioral manipulation.

In Romania, the species was also reported at *Carabus violaceus* (ELENA CHIRIAC, 1975).

In the specialized literature, we did not find this larval stage of the parasite reported to *M. melolontha*. As a result, we introduce new data on the host species into the information circuit.

Other mentions - in Southwest Anatolia, *Gordius* sp. at *Squalius fellowesii* (Cyprinidae) (HÜSEYIN ŞAŞI & DANIELA GIANNETTO, 2016), *Pterostichus inopinus* (Casey), *P. castaneus* Dejean, collected in the northwest of Oregon (GEORGE POINAR JR., JESSICA RYKKEN, LABONTE, 2004) and *G. aquticus* at *Pterostichus melas* Creutzer (Carabidae) (V. SIEBOLD, 1843, *In:* GEORGE POINAR JR. *et al.*, 2004).

Moreover, for each type of interspecific relationship, there are presented the ecological particularities of the coleopteran host and the phoretic parasite / predator / organism. There are rendered aspects about their distribution and host species, as well as the dependence among the parasite, the host and the type of food.

We conclude that the parasites and predators found in Coleoptera consume the various stages of their biological development either in larvae - the exoendoparasite *Dialomermis leioderma* having as host the larva of *Cybister (Scaphinectes) lateralimarginalis*; the predatory pentatomid *Zicrona caerulea* of the *Melasoma populi* larva, as well as **exoparasite adults** - *Bauveria bassiana, Metarizium anisoplie*; ? deutonymph of mites – *Uropoda* sp. (in the females of *Copris lunaris*), *Hypoaspis* sp. - *Oryctes nasicornis*; *Macrocheles* sp. (in males of *Onthophagus (P.) vacca*, *O. taurus*) and **endoparasites** *Ascarops strongilina, Macrachantorincus hirudinaceus* in adults of *Aphodius fimetarius* and *Melolontha melolontha*.

In the study we have introduced, as an interspecific relationship type, the following hosts for parasites, predators and phoretic organisms, all new for Dolj County, Romania: *Beauveria bassiana* - *Cerambyx cerdo; Zicrona caerulea* - *Melasoma populi; Metarhizium anisopliae, Hypoaspis* sp.- *Oryctes nasicornis; Uropoda copridis* - *Copris lunaris; Uropoda* sp. - *Copris lunaris.*

Moreover, we identified certain species of Coleoptera – hosts for parasites and phoretic organisms, new for Dolj County: *Drilomermis leioderma* - *Cybister (Scaphinectes) lateralimarginalis*; *Ascarops strongylina* - *Aphodius fimetarius*; *Gordius aquaticus, Macracanthorhynchus hirudinaceus* - *Melolontha melolontha; Macrocheles* sp. - *Onthophagus (Palaeonthophagus) vacca, O. taurus.*

The chapter ends with **the synthesis of the results regarding the interspecific relations of parasitism, predation, and phoresy at the Coleoptera species found in ecosystems on the territory of Dolj County** presented in the form of a table containing the 16 host Coleoptera, the identified parasite / predator / mite, the research sites and collection date.

The last chapter, **chapter 7**, deals with the results of the research only from an ecological point of view, issuing ecological considerations and practical recommendations on the role of Coleoptera and interspecific relations in nature and in the conservation of biodiversity. Based on the two major environmental criteria, we analyzed the research material and came to the following conclusions:

No.	Type of relationship	Parasite/ predator/phoretic organism	Host
1.	**interspecific relationships established on the direct effect criterion**		
	unilaterally positive and unilaterally negative relationships (+ -)		
1.1.	parasitism (+-)	*Drilomermis leioderma* Poinar & Petersen, 1978	*Cybister (Scapinectes) lateralimarginalis* (De Geer 1774)
		Beauveria bassiana (Bals.-Criv.) Vuill. 1912	*Cerambyx cerdo* Linnaeus, 1758
		Metarhizium anisopliae (Metchnikoff) Sorokin	*Oryctes nasicornis* Linnaeus 1758
		Ascarops strongylina Rudolphi, 1819	*Aphodius fimetarius* (Linnaeus 1758)
		Macracanthorhynchus hirudinaceus (Pallas 1781)	*Melolontha melolontha* (Linnaeus 1758)
		Gordius aquaticus Linnaeus 1758	*Melolontha melolontha* (Linnaeus 1758)
1.2.	predation (+-)	*Zicrona caerulea* (Linné., 1758)	*Melasoma populi* (Stephens, 1834)
2.	**interspecific relationships established based on their role in the lives of the populations criterion**		
	distribution, phoretic or transport relationships	*Hypoaspis* sp. G. Canestrini, 1884	*Oryctes nasicornis* (Linnaeus1758)
		Uropoda copridis Oudemans, 1916	*Copris lunaris* (Linnaeus1758)
		Macrocheles muscaedomesticae (Scopoli, 1772)	*Pentodon idiota idiota* Herbst, 1789
		Macrocheles sp. (Willmann, 1939)	*Onthophagus (P.) vacca* (Linnaeus 1767)
			Onthophagus Taurus (Schreber, 1759)

The chapter also contains the *synecological analysis of the coleopteran populations*, as well as the effects of anthropogenic activity. Taking into account that the coleopteran species are different and are collected from different research sites, we have used for exemplification two of the most numerous species: *Pentodon idiota idiota* and *Onthophagus (P.) vaca*.

Following the interpretation of the analytical indices, it can be concluded that the species *Pentodon idiota idiota* is an **accidental species** in the locality of Secui. This species belongs to class **D5 - eudominant species** and class **W4** that contains **characteristic species (constant)** compared to *Onthophagus vaca* – **accessory species** belonging to class **D5 - eudominant species**, class **W4**, which contains **characteristic species (constants)**.

CONCLUSIONS

1. The collection of the research material was made seasonally, from April to September, during 2009-2017, and there were investigated 782 specimens of Coleoptera from 40 localities, out of which 16 are new research sites.
2. The Coleoptera fauna collected from these localities comprises 10 species that systematically belong to the Coleoptera order and fall into 7 families. Most of them belong to the Scarabaeoidea family.
3. Consequently, in the period 2009-2017, there were identified 7 species of parasites, one species of predators, 6 species of mites belonging to 2 kingdoms, 5 phyla, 10 classes, 10 orders, 9 families, 9 suborders, 10 genera (2 saprophytic fungi: *Beauveria bassiana* and *Metarhizium anisopliae;* one acanthocephalan - *Macracanthorhynchus hirudinaceus;* 6 mites - *Uropoda copridis, Uropoda* sp., *Macrocheles muscadomestcaee, Macrocheles punctilatus, Macrocheles* sp., *Hypoaspis* sp.; one pentatomid - *Zicrona caerulea;* 2 nematodes - *Drilomermis leioderma; Ascarops strongylina* and one Nematomorpha - *Gordius* sp.).
4. During the research, it has resulted that the saprophytic fungi were the most simple to determine, while mites were the most difficult.
5. Among the species of Coleoptera with the most numerous interspecific relationships, we mention those belonging to the Scarabaeinae family with 6 species, followed by Dynastinae with 4 species, Melolonthinae with 2 species, and Aphodiinae, Chrysomelinae, Cerambycinae, Dytiscinae with one species each.
6. There were identified three types of intraspecific relationships: **parasitism** (*Beauveria bassiana* at *Cerambyx cerdo; Metarhizium anisopliae* at *Oryctes nasicornis; Drilomermis leioderma* at *Cybister (Scaphinectes) lateralimarginalis*; *Ascarops strongylina* at *Aphodius fimetarius*; *Gordius aquaticus, Macracanthorhynchus hirudinaceus* at *Melolontha melolontha*); **predation** (*Zicrona caerulea* la *Melasoma populi)* and **phoresy** (*Hypoaspis* sp. at *Oryctes nasicornis; Uropoda copridis* at *Copris lunaris; Uropoda* sp. at *Copris lunaris, Macrocheles* sp. at *Onthophagus (Palaeonthophagus) vacca, O. taurus, Macrocheles muscaedomesticae* at *Pentodon idiota idiota; Macrocheles punctillatus* at *Onthophagus (Palaeonthophagus) vacca).*
7. Based on personal observations from the specialized literature, there resulted a close relationship between the trophic regime represented by larvae (*Cybister (S.) lateralimarginalis*; *Melasoma populi*), but also by adults (*Aphodius fimetarius*; *Melolontha melolontha*), temperature (*Cerambyx cerdo; Oryctes nasicornis*) and the presence of parasites as these are two essential factors for survival and perpetuation.
8. In other words, the particularities of phoresy at mites include the active search of a host, the recognition of attachment signs and the host specificity, the state of silence, the recognition of the abandonment signs of the host and, if necessary, synchronization with the lifecycle of the host.
9. As a result, several dispersion mechanisms are used at different stages of development. Mites continually reproduce in an appropriate environment, but they disperse when certain environmental factors adversely affect their presence in the habitat (KRANTZ, 1999).
10. Phoresy is important for the maintenance of species that can act as predators or parasites at different stages of development and serves to define the strategy of survival of mutualistic, predatory and parasitic species (TATYANA SACCHI & PIRES DO PRADO, 2004).
11. The present study is an important contribution to the knowledge, identification and distribution the parasites of some coleopteran species in Dolj County, given the concrete results obtained on the 10 species of Coleoptera that are hosts for species of parasites, predators and mites.
12. The chosen research method may be extrapolated to other areas of study given that, under favorable conditions, the pathogenicity of some of the presented parasites may increase greatly, having a potential zoonotic character.
13. There are also positive aspects of this cohabitation; for example, parasitic fungi have been and can be used as control agents for harmful species, or the predator of *Zicrona caerulea* can be used to reduce the number of defoliating species.
14. Indicator species, such as *Cybister* (*Scapinectes*) *lateralimarginalis*, are a useful management tool and can help us delineate ecoregions, indicate environmental status, discover an outbreak of a disease or the level of pollution of the area, or climate change. In a sense, they can be used as an

"early warning system". Indicator species must also be accompanied by an in-depth study of the area and how this species fit in the rest of the ecosystem.

15. By mastering science and technology, man has become a dominant species in the ecosphere, being able to transform the environment, adapt it to its needs, while in case of other species the process is inverse - the species change by adapting to the environment (BOTNARIUC & VĂDINEANU, 1982). At the same time, however, man remains a component (a subsystem) within the ecosphere system. Changes in certain features of the ecosphere are also reflected upon himself.
16. The concept on which the entire process of environmental protection is organized starts from the understanding of the fact that natural factors (water, air, soil, subsoil, vegetal and animal organisms) are in close and permanent interaction, being systemically integrated in the highest level of organization of environmental structures – the ecosphere. Consequently, the set of environmental protection standards and means must be unitary (CIOBOIU-CODOBAN, 2005).

REFERENCES

1. LILA GIMA. 2018a. *Preliminary data regarding interspecific relationships beetle species collected from different ecosystems met in DOLJ county in 2017*. *Note 1.* Museum of Oltenia Craiova. Oltenia Journal for Studies in Natural Sciences. Thomson Reuters - Zoological Record; CAB Abstracts; EBSCO; C.N.C.S.I.S. B+ category. – in press.
2. LILA GIMA. 2018b. *Preliminary data regarding interspecific relationships beetle species collected from different ecosystems met in DOLJ county in 2017. Note 2.* Museum of Oltenia Craiova. Oltenia Journal for Studies in Natural Sciences. Thomson Reuters - Zoological Record; CAB Abstracts; EBSCO; C.N.C.S.I.S. B+ category.– in press.
3. LILA GIMA. 2017a. *Preliminary data regarding beetle parasite species collected from different ecosystems met in DOLJ county in 2017*. Museum of Oltenia Craiova. Oltenia Journal for Studies in Natural Sciences. Thomson Reuters - Zoological Record; CAB Abstracts; EBSCO; C.N.C.S.I.S. B+ category. **32**(1): 77-81;
4. LILA GIMA. 2017b. *Preliminary data regarding beetle parasite species collected from different ecosystems met in DOLJ county in 2017*. Museum of Oltenia Craiova. Oltenia Journal for Studies in Natural Sciences. Thomson Reuters - Zoological Record; CAB Abstracts; EBSCO; C.N.C.S.I.S. B+ category. **32**(2): 67-71;
5. LILA GIMA. 2015a. *Preliminary data regarding beetle parasite species collected from different ecosystems met in Dolj County in 2014-2015*. Museum of Oltenia Craiova. Oltenia Journal for Studies in Natural Sciences. Thomson Reuters - Zoological Record; CAB Abstracts; EBSCO; C.N.C.S.I.S. B+ category. 31(2): 89-94;
6. LILA GIMA. 2015b. *Preliminary data regarding beetle parasite species collected from different ecosystems met in Dolj County in 2014-2015*. Museum of Oltenia Craiova. Oltenia Journal for Studies in Natural Sciences. Thomson Reuters - Zoological Record; CAB Abstracts; EBSCO; C.N.C.S.I.S. B+ category. **31**(1): 75-82;
7. LILA GIMA. 2014. *Contribution to the knowledge of research on beetle parasite fauna (Insects). Note 2.* Museum of Oltenia Craiova. Oltenia Journal for Studies in Natural Sciences. Thomson Reuters - Zoological Record; CAB Abstracts; EBSCO; C.N.C.S.I.S. B+ category. **30**(2): 105-120;
8. LILA GIMA. 2013. *Contributions to the knowledge of research on beetle parasite fauna (Insects). Note 1.* Museum of Oltenia Craiova. Oltenia Journal for Studies in Natural Sciences. Thomson Reuters - Zoological Record; CAB Abstracts; EBSCO; C.N.C.S.I.S. B+ category. **29**(2): 172-183;
9. LILA GIMA. 2012. *Data regarding the presence of Cybister (scaphinectes) lateralimarginalis (DEGEER 1774) (Insecta: Coleoptera: Dytiscidae) in the entomological fauna of Dolj county (Romania).* Museum of Oltenia Craiova. Oltenia Journal for Studies in Natural Sciences. Thomson Reuters - Zoological Record; CAB Abstracts; EBSCO; C.N.C.S.I.S. B+ category. **28**(2): 63-66;
10. LILA GIMA. 2011. *A surwey of known parasitoides of family Cerambycidae (Insecta: Coleoptera: Chrysomeloidea).* Museum of Oltenia Craiova. Oltenia Journal for Studies in Natural Sciences. Thomson Reuters - Zoological Record; CAB Abstracts; EBSCO; C.N.C.S.I.S. B+ category. **27**(2): 115-122;

11. MOGOȘEANU GIMA. 2010. *Data regarding the presence of the Families Dytiscidae and Hydrophilidae (Insecta: Coleoptera) in the entomological fauna of Oltenia (Romania) (I).* Museum of Oltenia Craiova. Oltenia Journal for Studies in Natural Sciences. Thomson Reuters - Zoological Record; CAB Abstracts; EBSCO; C.N.C.S.I.S. B+ category. **26**(2): 133-136;
12. CHIMIȘLIU CORNELIA & MOGOȘEANU GIMA. 2009. *Considerations on the Dynastidae Family (Insecta: Coleoptera: Scarabaeoidea), from the fauna of Oltenia, Romania.* Museum of Oltenia Craiova. Oltenia Journal for Studies in Natural Sciences. Thomson Reuters - Zoological Record; CAB Abstracts; EBSCO; C.N.C.S.I.S. B+ category. **25**: 125-128;
13. PANIN S. 1957. *Insecta. Coleoptera – Familia Scarabaidae II.* Edit. Acad. R. P. R. **10**(4). 315 pp., 36 plş.
14. TATYANA SACCHI CARMONA RODRIGUEIRO & ANGELO PIRES DO PRADO. 2004. *Macrocheles muscaedomesticae (Acari, Macrochelidae) and a species of Uroseius (Acari, Polyaspididae) phoretic on Musca domestica (Diptera, Muscidae): effects on dispersal and colonization of poultry manure.* Iheringia, Sér. Zool., Porto Alegre, **94**(2): 181 pp., 30 de junho de. file:///C:/Users/asus/Desktop/anexe%20doc/ lucrare%20 tatiana.pdf. (accesed: april, 2017).